Design and synthesis of a consensus signal sequence that inhibits protein translocation into rough microsomal vesicles

Brian M. AUSTEN, John HERMON-TAYLOR, Mustak A. KADERBHAI and David H. RIDD
Department of Surgery, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, U.K.

Most signal sequences are found to vary considerably in length and primary sequence, but possess some common structural features. Analysis of known signal sequences has led to the design of a 19-residue sequence that, although not a naturally occurring signal, possesses the structural features that commonly occur in pre-proteins. This peptide has been synthesized by solid-phase methods, and has been shown to inhibit, in a concentration-dependent manner, the processing *in vitro* of nascent pre-prolactin, pre-forms of pancreatic digestive enzymes, and pre-placental lactogen. The peptide acts at the cytoplasmic surface of microsomal vesicles added to the protein translation system, preventing translocation of the nascent chains to the lumenal space of vesicles where signal peptidase normally cleaves to remove the signal from nascent pre-proteins.

The amino acid sequences of more than 50 *N*-terminal extensions (signal sequences) found on the nascent chains or initially-translated pre-cursors of secreted or membrane-bound proteins from prokaryotic and eukaryotic sources are known (Blobel *et al.*, 1979; Chan *et al.*, 1979; Austen & Ridd, 1981). Signal sequences are believed to assist transfer of proteins across membranes, and in eukaryotic cells are thought to act by enabling the nascent chain–polysomal complex to bind to an 11 S ribonucleoprotein complex (Walter & Blobel, 1981), which in turn binds to a specific protein, termed docking protein, associated with the membrane of the endoplasmic reticulum (Meyer *et al.*, 1982). These interactions are followed by vectorial transfer of nascent chains into the lumen of this organelle.

Although no obvious primary sequence homologies are apparent among known signal sequences, a number of common structural features have been identified (Austen, 1979; Austen & Ridd, 1981; Blobel *et al.*, 1979; Garnier *et al.*, 1980; Chan *et al.*, 1979). Signal sequences commonly possess an uneven distribution of charged and uncharged residues, so that there exists a hydrophobic core of at least nine consecutive uncharged residues, bordered by basic amino acid residues at the *N*-terminus, and basic and acidic residues at the *C*-terminus. There is specificity in the region at which proteolytic cleavage takes place to give rise

to a mature protein, and predictions of secondary structure have demonstrated high potential for adopting α-helical structure in the central hydrophobic core, and β-turns close to the cleavage sites. In order to test the importance of these common structural features in effecting translocation of nascent secretory proteins into the endoplasmic reticulum, we have synthesized a peptide which is not a naturally occurring one, but represents a consensus of known signal sequences. This peptide has been tested for its activity in inhibiting translocation of nascent pre-proteins in cell-free protein translation systems *in vitro* into dog pancreatic microsomal vesicles present during translation.

Materials and methods

Materials

Dichloromethane (Fisons, Loughborough, U.K.), dried and distilled grade, was stored over molecular sieve (4A). Dimethylformamide (Fisons) and di-isopropylethylamine (Fluorochem, Glossop, Derbyshire, U.K.) were distilled from ninhydrin *in vacuo*. Trifluoroacetic acid (Koch-Light, Slough, Berks., U.K.) was redistilled. Protected amino acid derivatives, from Vega Biochemicals, Tucson, AZ, U.S.A., were tested for purity on t.l.c. and recrystallized if necessary. [^{35}S]-Methionine (>800 Ci/mmol) and [4,5^{3}H]leucine were from Amersham International, Amersham, Bucks., U.K. Synthetic γ-endorphin and human parathyroid hormone (residues 28–48) were obtained from Cambridge Research Biochemicals,

Cambridge, U.K. Chloromethylated polystyrene (1% cross-linked SX1, 200–400 mesh, 1.29 me-quiv./g) from Bio-Rad, Watford, Herts., U.K., was substituted with Boc-Glu(O-benzyl) as its caesium salt (Gisin, 1973) to a level of 0.65 mmol/g, and additional reactive groups were blocked by treatment with benzoyl chloride (0.2 ml/g) and pyridine (0.18 ml/g). Aminopeptidase M was from Rohm, Darmstadt, Germany, and chymotrypsin and tosyl-phenylalanylchloromethane ('TPCK')-treated trypsin from Millipore, Freehold, NJ, U.S.A. Oligo(dT)–cellulose and biochemicals used in cell-free translation were from Sigma, Poole, Dorset, U.K., and X-ray film from Kodak, Hemel Hempstead, Herts., U.K. RNA from human placenta was obtained from NEN Chemicals, Dreieich, Germany. All other materials were of the highest grade available.

Peptide synthesis

The 19-residue consensus peptide and a control pentapeptide, Leu-Ala-Tyr-Val-Ala, were synthesized on solid phase (Erickson & Merrifield, 1976). All amino acids were protected at the α-amino positions with Boc groups, and the following side chain protectants were used: Lys, N-2-chlorobenzyloxycarbonyl; Asp, O-benzyl; Cys, S-4-methylbenzyl; Ser, O-benzyl; Tyr, O-2,6-dichlorobenzyl. After two alternating washes with dichloromethane and propan-2-ol, three washes with dichloromethane, deprotection with 50% (v/v) trifloroacetic acid in dichloromethane for two cycles, one of 5 min, the next of 25 min, was performed. Then, after four washes with dichloromethane, the resin was neutralized with three washes of 5% (v/v) di-isopropylethylamine in dichloromethane. After four washes with dichloromethane, two with propan-2-ol and five with dichloromethane, protected amino acids (4-fold molar excess) were coupled with dicyclohexyl-carbodi-imide (3-fold molar excess) for 2 h. Coupling was monitored with a ninhydrin test (Kaiser *et al.*, 1970). The coupling step was repeated at step 8 (Val), step 9 (Tyr), step 11 (Leu), step 12 (Ala), step 13 (Leu) and step 14 ([4,5-^{3}H]Leu). At step 12, the repeat coupling was performed with the addition of a 3-fold molar excess of 1-hydroxybenzotriazole in dimethylformamide. Boc-[4,5^{3}H]Leu was incorporated at step 13 to provide a convenient radioactive marker. After coupling, the resin was washed with alternate cycles of dichloromethane (nine times), dimethylformamide (twice), and methanol (twice), and any unreacted peptide terminated by reaction with a 5-fold molar excess of N-acetylimidazole in dichloromethane.

Peptides were removed from resin supports and deprotected with HF, freshly distilled from CoF$_3$, for 1 h at 0°C in the presence of anisole and ethane-dithiol, and extracted into 30% (v/v) acetic acid. The acetic acid was removed by rotary evaporation, and a portion of the residue arising from synthesis of the 19-residue peptide (0.96 mmol) taken up in 0.1 M-NH$_4$HCO$_3$ (pH 8.9)/6 M-guanidinium chloride (26 ml), and incubated for 48 h with sodium sulphite (10 mmol) and sodium tetrathionate (5 mmol). Acetic acid was then added to give 30% (v/v), and the sulphonylated peptide was purified by gel filtration on Sephadex G-50, ion-exchange chromatography on SP (sulphopropyl)-Sephadex, and finally reverse-phase h.p.l.c. on SynChroPak RP-P (Synchron, Linden, IN, U.S.A.) in 25% (v/v) acetic acid adjusted to pH 2.5 with 1 M-NH$_4$OH, eluting with a linear gradient of acetonitrile. The pentapeptide was purified by gel filtration on Sephadex G-25 in 30% (v/v) acetic acid.

The sulphonylated peptide (60 nmol) in formic acid (0.05 ml; 70%, v/v) was cleaved by incubation with CNBr (30 μmol) overnight at room temperature. The incubate was diluted with water and lyophilized. Sulphonylated peptide (60 nmol) was also digested with trypsin (7 μg) in 0.1 M-NH$_4$HCO$_3$ (pH 8.0) (0.2 ml) for 12 h at 22°C, and lyophilized after addition of water. Peptide fragments were separated by t.l.c., eluted from the strip with 30% (v/v) acetic acid, and identified by amino acid analysis after acid hydrolysis. The CNBr fragments and tryptic fragments were added to cell-free translation systems after desulphonylation as described below.

Analyses of synthetic peptides

Small samples of peptides were hydrolysed *in vacuo* in 6 M-HCl containing 0.01% mercaptoacetic acid and 0.01% phenol at 110°C for 18 h. Peptides (70 nmol) were digested with aminopeptidase M (50 μg) and chymotrypsin (5 μg) for 2 h 30°C in 0.1 M-NH$_4$HCO$_3$ (0.1 ml). Amino acid analyses were performed on an LKB 4400 amino acid analyser, using a standard citrate programme for elution. Peptides were subjected to high-voltage paper electrophoresis for 30 min at 3 kV at pH 1.9. T.l.c. was performed on cellulose in butan-1-ol/water/pyridine/acetic acid (15:12:10:3, by vol.)

Cell-free translations

Poly (A)-containing RNA was isolated from dog pancreas and bovine pituitaries by extraction into guanidine thiocyanate (Chirgwin *et al.*, 1979) and chromatography on oligo(dT)–cellulose (Bantle *et al.*, 1976), and translated in a nuclease-treated rabbit reticulocyte lysate (Pelham & Jackson, 1976). Dog rough microsomal membranes were prepared, and portions stripped by treatment with micrococcal ribonuclease, while other portions

were stripped by EDTA, as described by Scheele *et al.* (1980). The integrity of vesicles was examined under the electron microscope. Before adding to cell-free translations, synthetic peptides were incubated in 0.2M-dithiothreitol/20mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid]/KOH (pH 7.4) at concentrations of 50mM at 37°C for 2h to remove the sulphonyl protecting group, then lyophilized. Desulphonylated peptides were redissolved in 20mM-Hepes/KOH (pH 7.4)/2mM dithiothreitol, their concentration measured by radioactivity, and then added to 3 vol. of stripped microsomes to give them a concentration of $25 A_{260}$ units/ml together with concentrations of peptide up to 250 μM. After 3min at 22°C, 4 μl aliquots of the membranes with peptide were transferred to rabbit reticulocyte translation mixtures (16 μl) that 1min previously had been initiated in protein synthesis by addition of poly(A)-containing RNA. The final concentration of microsomes within the cell-free system was $5 A_{260}$ units/ml, poly(A)-containing RNA concentrations were 45 μg/ml (bovine pituitary) or 70 μg/ml (dog pancreas), peptide concentrations ranged between 3 and 50 μM, and other constituents were [^{35}S]methionine (750 μCi/ml), ATP (0.94mM), GTP (0.18mM), KCl (80mM), MgCl$_2$ (1.5mM). Hepes/KOH (pH 7.4; 7.5mM) dithiothreitol (2mM), creatine phosphate (9.4mM), creatine phosphokinase (0.23 μg/ml), unlabelled amino acids without methionine (20–120 μM) and haemin (12 μM), and translation was continued for 60min at 30°C. Human placental RNA was translated in reticulocyte lysate supplied by NEN Chemicals as described by the supplier.

To examine the state of protection of ^{35}S-labelled products at the end of translation, samples were incubated further at 0°C with a mixture of chymotrypsin (100 μg/ml) and trypsin (100 μg/l) in the presence or absence of sodium deoxycholate (1%, w/v). Control samples were also incubated further without any additions. After 1h, Trasylol (5500 k.-i.u./ml) was added.

Proteins were analysed after addition of 3 vol. of 0.1M-Tris/HCl (pH 6.8), 10% (w/v) SDS and 1M-mercaptoethanol and boiling for 5min, by subjecting them to electrophoresis on polyacrylamide, followed by fluorography of dried gels (Laskey & Mills, 1975), and quantification on a Kratos model 3000 spectrodensitometer fitted with a computing integrator. Gels were either 15% (w/v) in polyacrylamide, or were gradients of 10–17.5%, as described in Figure legends.

Results

The structure of the consensus signal peptide is shown in Fig. 1. Amino acid analysis of the purified synthetic peptide (Table 1) and electrophoresis of the peptide on paper at pH 1.9 (R_{Asp} 1.12) indicated that the required peptide had been synthesized, and was homogeneous. Complete cleavage by CNBr into two peptides, residues 1–9 (R_{Asp} 1.12, R_F 0.79 on t.l.c.) and residues 10–19 (R_{Asp} 1.66, R_F 0.29 on t.l.c.), showed that the methionine residue was not oxidized. Tryptic digestion yielded three peptides, Lys-Lys (R_{Asp} 2.94) Ala-Asp-Lys-Glu (R_{Asp} 1.72) and a 13-residue peptide (residues 3–15 inclusive), as expected from its sequence.

The synthetic consensus peptide was tested for its effect on the processing of the products of translation of mRNA isolated from three different sources in translation systems *in vitro* derived from nuclease-treated rabbit reticulocyte lysates supplemented with stripped rough microsomes isolated
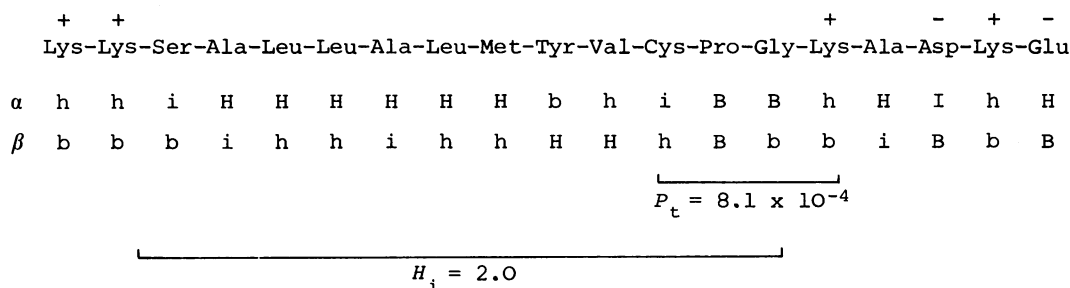
	+	+								+		-	+	-
Lys-Lys-Ser-Ala-Leu-Leu-Ala-Leu-Met-Tyr-Val-Cys-Pro-Gly-Lys-Ala-Asp-Lys-Glu

α	h	h	i	H	H	H	H	H	H	b	h	i	B	B	h	H	I	h	H
β	b	b	b	i	h	h	i	h	h	H	H	h	B	b	b	i	B	b	B

$$P_t = 8.1 \times 10^{-4}$$

$$H_i = 2.0$$

Fig. 1. *Structure of the consensus signal sequence*

The peptide sequence was designed by examination of common structural features occurring in 52 known signal sequences (Austen & Ridd, 1981). The influence of residues on predicted secondary structure: αH, strong α-former; αh, α-former; αI, weak α-former; αi, indifferent; αb, α-breaker; αB, strong α-breaker; βH, strong β-former; βh, β-former; βi, indifferent; βb, β-breaker; βB, strong β-breaker. Potential for β-turn (P_t) was calculated according to Chou & Fasman (1978). The average hydrophobicity (H_i) was derived as described by Segrest & Feldman (1974).

Table 1. *Amino acid analyses of consensus signal peptide*
N.D., not detected

Residue	Composition (mol/mol of peptide)		
	Acid hydrolysis (20 h)	Theoretical	Aminopeptidase/chymotrypsin (2 h)
Asp	1.03	1	0
Ser	0.90	1	0.98
Glu	1.11	1	0
Pro	0.98	1	0
Gly	1.07	1	0
Ala	2.99	3	1.84
Val	1.00	1	0.57
Met	0.97	1	0.72
Leu	2.88	3	2.16
Tyr	0.93	1	0.59
Lys	3.97	4	2.02
Cys	N.D.	1	0
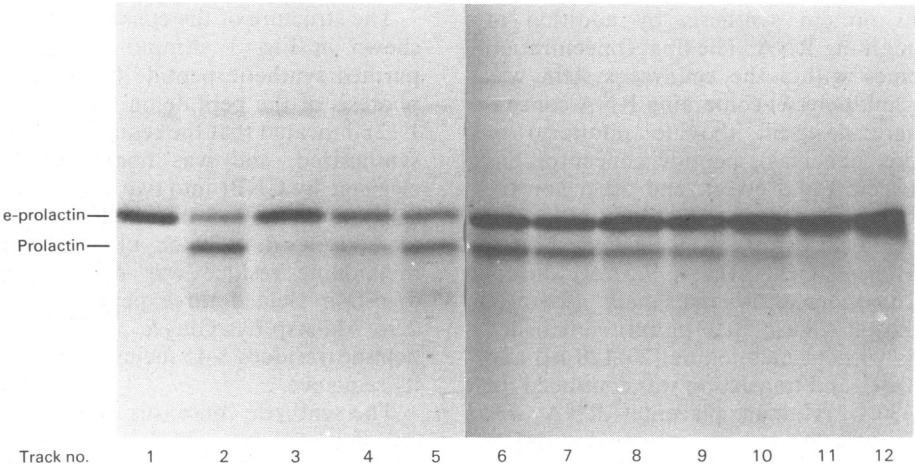
Fig. 2. *Blockage of processing of bovine pre-prolactin by consensus signal peptide*
Translation of bovine pituitary mRNA was performed in the presence of pancreatic microsomal membranes and peptide, and ^{35}S-labelled proteins were analysed by electrophoresis on polyacrylamide (15%) in SDS. Track 1, −EDTA membranes, −peptide; track 2, +EDTA membranes, −peptide; track 3, +EDTA membranes, +peptide (5 μM final concentration); track 4, +EDTA membranes, +peptide (25 μM); track 5, +EDTA membranes, +peptide (50 μM); track 6, +nuclease membranes, −peptide; track 7, +nuclease membranes, +peptide (3 μM); track 8, +nuclease membranes, +peptide (7.5 μM); track 9, +nuclease membranes, +peptide (12.5 μM); track 10, +nuclease membranes, +peptide (20 μM); track 11, +nuclease membranes, +peptide (35 μM); track 12, +nuclease membranes, +peptide (50 μM).

from dog pancreas. Under the electron microscope, rough microsomes were seen to consist entirely of vesicles from about 200 to 600 μm in diameter, studded with ribosomes. The latter were lost after treatment with EDTA, but the membranes retained their vesicular appearance. In the presence of EDTA-stripped rough microsomes, the major product of translation of bovine pituitary mRNA, preprolactin (Fig. 2, track 1), was converted into a form which migrates faster on SDS/polyacrylamide gels, and has been shown to be identical to the mature secreted form of prolactin (Shields & Blobel, 1977) (Fig. 2, track 2). The extent of processing determined by densitometry achieved with nuclease-treated microsomes (35%) was less than that achieved with EDTA-stripped microsomes (70%) (compare tracks 6 and 2). Processing of nascent pre-prolactin was inhibited in a dose-dependent fashion by 3–50 μM concentrations of the desulphonylated peptide with both EDTA-stripped rough microsomes (tracks 3–5) and nuclease-treated rough microsomes (tracks 7–12).

The peptide was also tested for its inhibitory action against processing of products of translation of mRNA obtained from two other sources. The

^{35}S-labelled products produced by translation of human placental mRNA in a rabbit reticulocyte lysate is shown in Fig. 3 (track 1). A major radio-labelled protein with an apparent M_r of 25000 was produced. A second high-M_r ^{35}S-labelled product seen on the gel (tracks 1–4) was also produced by reticulocyte lysates not programmed with exogenous mRNA, and results from ribosome-independent addition of [^{35}S]methionine to a pre-existing protein (Jackson & Hunt, 1983). When EDTA-stripped microsomes were present during translation, a smaller labelled protein was produced (track 2), which has previously been shown to correspond to authentic human placental lactogen (Szczesna & Boime, 1976). Consensus peptide inhibits this conversion progressively at concentrations of 24 μM (track 4) and 48 μM (track 3). ^{35}S-labelled products yielded by translation of dog pancreatic mRNA in a rabbit reticulocyte lysate are shown in Fig. 3 (track 5). Assignments have been made by comparison with the results of Scheele et al. (1980), and by changes yielded in the banding pattern by including enterokinase, (EC 3.4.21.9), a proteinase which selectively cleaves trypsinogen, in the translation mixture (Ridd et al., 1982). Products of M_r 55000 and 57000 are pre-amylase and pre-lipase, bands in the region of 45000–48000 include the pre-forms of procarboxy-peptidases plus the [^{35}S]methionine-labelled protein mentioned above and also seen in tracks 1–4,

and bands in the region 27000–33000 are the pre-forms of serine zymogens, of which the member of lowest M_r has been found to be cationic pre-trypsinogen (Ridd et al., 1982). A smaller protein of M_r 16000 is pre-prophospholipase. When EDTA-stripped microsomes were included in the translation, significant changes occurred in the banding pattern, and smaller proteins were produced, clearly seen in the production of cationic trypsinogen from pre-trypsinogen and prophospholipase from its pre-form (track 6). The addition of consensus signal peptide again caused inhibition of this change, being more potent at 48 μM (track 7) than 24 μM (track 8).

To investigate further the specificity of the action of the consensus sequence in blocking processing of pre-forms into mature forms of secreted proteins, effects of control peptides were studied. Peptides formed by cleavage of the consensus sequence by CNBr and trypsin, an additional hydrophobic pentapeptide, a hydrophobic peptide fragment from human PTH (residues 28–48), and the 17-residue peptide γ-endorphin were tested for their efficiency at inhibiting the co-translational processing of bovine pre-prolactin. The extent of inhibition of processing, measured by densitometry of X-ray films, in the presence of various peptides are shown in Fig. 4. Whereas 50 μM of the whole sequence completely prevented processing, the tryptic fragments together at 50 μM
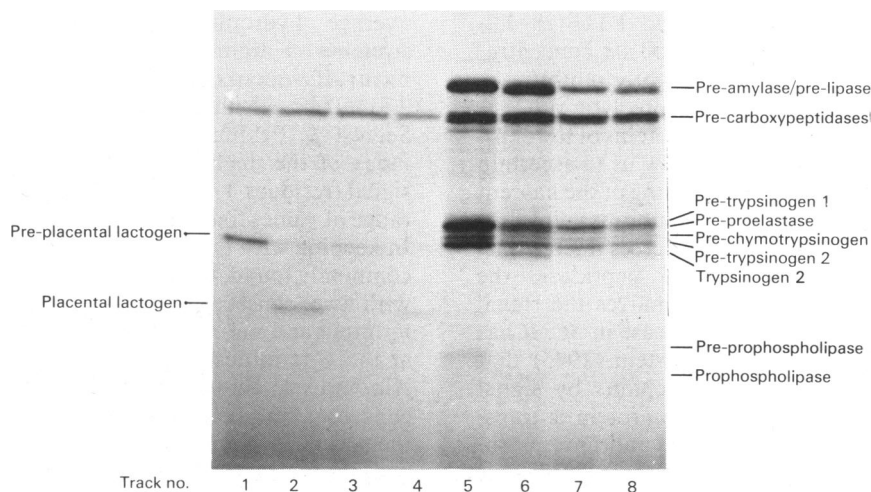

Fig. 3. *Blockage of processing of human placental lactogen and dog pancreatic pre-digestive enzymes by consensus signal peptide*

The effects of desulphonylated signal peptide on processing achieved in the presence of EDTA-stripped microsomes were examined in translation systems programmed with full-term human placental RNA (25 μg/ml) (tracks 1–4) or dog pancreatic RNA (70 μg/ml) (track 5–8) as described in the Materials and methods section. ^{35}S-labelled proteins were separated on gradient polyacrylamide gels (10–17.5, w/v) in SDS. Tracks 1 and 5 show ^{35}S-labelled proteins translated by mRNA alone, tracks 2 and 6 the ^{35}S-labelled proteins yielded in the presence of microsomes, and other tracks the effects of signal peptide at 24 μM (tracks 3 and 7) or 48 μM (tracks 4 and 8) concentrations.
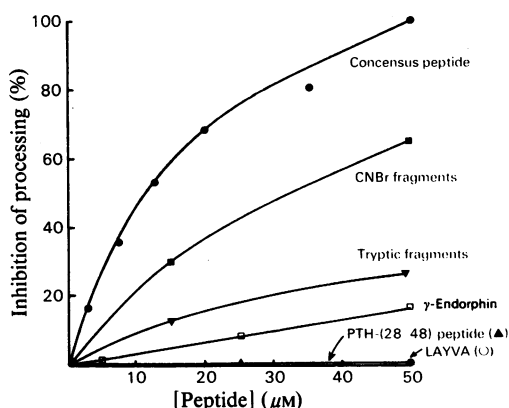
Fig. 4. *Effects on processing of bovine pre-prolactin by fragments of consensus signal peptide, and control peptides* γ-Endorphin, PTH-(28–48)-peptide, combined CNBr peptides, and tryptic peptides released by digestion of the consensus signal sequence, and a hydrophobic synthetic pentapeptide Leu-Ala-Tyr-Val-Ala (LAYVA), were incubated with nuclease-treated dog rough microsomes, and added to a translation system programmed with poly (A)-containing RNA from bovine pituitary as described in the Materials and methods section. ^{35}S-labelled proteins were separated by electrophoresis on polyacrylamide (15%, w/v) in SDS, fluorographed, and quantified by densitometric scanning of the X-ray film.

reduced processing by two-thirds, and the CNBr fragments by one-fifth. The hydrophobic pentapeptide Leu-Ala-Tyr-Val-Ala and PTH-(28–48)-peptide were not inhibitory at 50 μM concentrations, while γ-endorphin was slightly inhibitory.

The observed inhibition of proteolytic processing, which gives rise to mature proteins of lower M_r than the pre-forms, does not allow us to ascertain whether the peptide inhibits binding of the nascent pre-proteins to microsomal membranes, inhibits passage of nascent pre-proteins across microsomal membranes, or inhibits signal peptidase, the enzyme which proteolytically removes the signal sequence and gives rise to a decrease in M_r. It has been shown (Blobel & Dobberstein, 1975) that proteolysis of nascent protein chains by signal peptidase only occurs if first the protein is translocated across the microsomal membrane, presumably because the active site of the peptidase is confined to the lumenal surface of microsomes. Thus, post-translational limited proteolysis was performed in order to ascertain the location of the precursor that accumulates in the presence of signal peptide. The results are shown in Fig. 5. In the presence of the consensus sequence, inhibition of processing occurs (track 1). In the presence of deoxycholate, chymotrypsin and trypsin added at

the end of translation cause disappearance of both pre-prolactin and its mature form (track 2). In the absence of detergent, almost all of the pre-form disappears, while the mature form is resistant (track 3). The results of the same conditions of limited proteolysis applied to ^{35}S-labelled proteins translated in the absence of inhibition by peptide (tracks 4, 5 and 6) shows that again the mature form is resistant to proteolysis in the absence of detergent, but that the pre-form is susceptible. Tracks 7, 8 and 9 show the results obtained when post-translational proteolysis is performed on unprocessed [^{35}S]pre-prolactin translated in the absence of microsomes. As expected, the pre-form is completely degraded. Thus, because the pre-form is still accessible to proteinases when translated in the presence of peptide, the predominant mode of inhibition occurs on the cytoplasmic surface of the microsomal residues, although additional inhibition of signal peptidase cannot be excluded. The results also show that, although the peptide contains both hydrophobic and hydrophilic regions, it does not cause inhibition by detergent-like disruption of the membranes which would expose the processed form of prolactin to exogenous proteinases.

Discussion

The 19-residue consensus sequence was devised by consideration of common structural features contained in known signal sequences, as described previously (Austen & Ridd, 1981). Calculated average hydrophobicity over the uncharged stretches of amino acid residues found in many naturally-occurring signal sequences was 2.31 ± 0.49 calculated according to the index of Segrest & Feldman (1974). The hydrophobicity index of the uncharged stretch in the consensus signal (residues 3–14) was 2.0, in keeping with the range of values found in natural sequences (Fig. 1). In keeping with the double amphiphilic structures commonly found, the consensus peptide was made with two consecutive lysine residues at the N-terminus and a mixture of basic and acidic residues at the C-terminus (Fig. 1). A sequence Gly-Lys-Ala-Asp was chosen at the C-terminal end of the consensus peptide as this would be in keeping with the specificity found at the region at which signal peptidase cleaves. This has been summarized as Aaa-Xaa-Baa-Yaa (Austen & Ridd, 1981), where Yaa is the N-terminus of the mature protein, Xaa is any residue, Aaa is an uncharged residue, and Baa is an uncharged residue with a small side chain. The consensus sequence would thus be anticipated to offer a potential cleavage site to signal peptidase between residues 16 and 17. An N-terminal methionine residue was not included, as this is
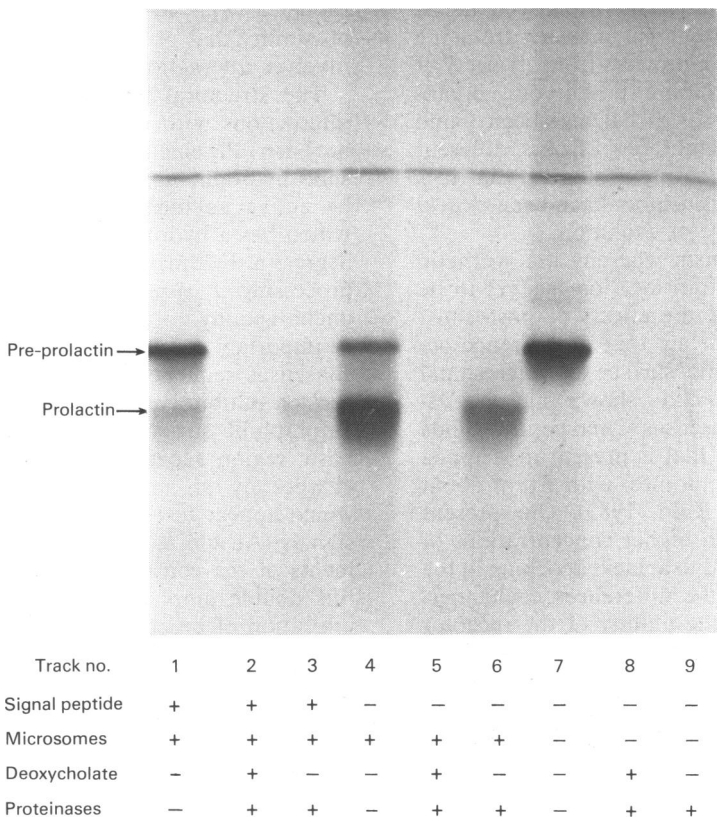
Track no.	1	2	3	4	5	6	7	8	9
Signal peptide	+	+	+	−	−	−	−	−	−
Microsomes	+	+	+	+	+	+	−	−	−
Deoxycholate	−	+	−	−	+	−	−	+	−
Proteinases	−	+	+	−	+	+	−	+	+

Fig. 5. *Examination of the state of protection of processed bovine prolactin to post-translational proteolysis*
Three translations of bovine pituitary poly(A)-containing RNA were performed, one in the presence of EDTA-stripped microsomes plus desulphonylated signal peptide at $50\,\mu$M final concentration (tracks 1–3), one in the presence of microsomes (tracks 4–6) and one with no additions apart from compensating amounts of Hepes/KOH buffer or 0.25 M-sucrose (tracks 7–9), as described in the Materials and methods section. After translation, each incubation mixture was divided into three; one part was further incubated with proteinases and detergent (tracks 2, 5 and 8), one part with proteinases alone (tracks 3, 6 and 9), and one with no additions (tracks 1, 4 and 7). After addition of Trasylol, ^{35}S-labelled proteins were separated on polyacrylamide (15%, w/v) in SDS.

known to be removed from nascent polypeptides after about 20 residues have been biosynthesized (Palmiter *et al.*, 1978).

The 19-residue peptide we have synthesized has been shown to compete effectively with naturally occurring signal sequences contained in bovine pre-prolactin, human pre-placental lactogen and the pre-forms of dog pancreatic digestive enzymes, leading to inhibition of processing of these pre-forms *in vitro*. The concentration at which it exerts inhibition, i.e. from 3 to $50\,\mu$M, is similar to the concentrations at which a synthetic peptide encompassing the pre- and pro-parts of pre-preparathyroid hormone was found to inhibit processing of hormone pre-proteins *in vitro* (Majzoub *et al.*, 1980), and those at which a peptide resembling the signal of dog pretrypsinogen has been found to inhibit processing of dog pancreatic pre-proteins

(Austen & Ridd, 1981). The fact that the nineteen-residue peptide represents a consensus of known signal peptides, yet acts in a similar way to peptides that are naturally-occurring sequences, shows that it is the common structural features incorporated that are important for translocation, and not precise, highly conserved primary sequences of the type originally discovered in the signal sequences of dog pancreatic zymogens (Devillers-Thiery *et al.*, 1975).

In keeping with this idea is the demonstration that the consensus sequence inhibits with similar potency the processing of pre-proteins of three different species from three different types of cells. Thus, it is unlikely that each cellular type is equipped with its own subset of microsomal receptors or other proteins involved in translocation, but rather that very similar protein trans-

locators exist in different cells. Moreover, it has been shown that microsomal membranes from one cell type can process pre-proteins from a variety of different sources, including prokaryotic organisms (Muller *et al.*, 1982). It has also been found that microsomal membranes from different sources process the same pre-protein so that it is likely that protein translocators have been closely conserved in the course of evolution.

The precise mechanism whereby the synthetic signal peptide blocks translocation has yet to be determined. Studies of the effects of post-translational proteolysis indicate that interference occurs on the cytoplasmic surface of microsomal vesicles. Recent studies have shown that an ^{125}I-labelled form of the consensus signal peptide binds to a saturable receptor that is present in stripped rough microsomal membranes, with K_D of about $1 \times 10^{-7} M$ (Austen & Ridd, 1983). Our present studies show that much higher concentrations of this peptide are required to achieve blocking in the translocation system; the differences could, perhaps, be explained if the affinity of the receptor was altered by components present in the lysate translation system. Alternatively, the high-affinity binding sites discovered with ^{125}I-labelled signal peptides may not be involved in translocation. We have not yet been able to demonstrate direct binding of the consensus signal peptide to the 11S ribonucleoprotein complex isolated by Walter & Blobel (1981), although we cannot exclude the possibility that observed inhibition of processing involves interaction with this complex.

The structural features that are essential for interactions with microsomal translocators have not been fully elucidated. However, it is clear that a short hydrophobic sequence would not necessarily be active as the peptide Leu-Ala-Tyr-Val-Ala, which has a hydrophobicity index, as defined by Segrest & Feldman (1974), of 2.6, does not block processing *in vitro*. A relatively long section of uncharged, hydrophobic residues would appear to be important, as cleavage in the central core of the consensus sequence by CNBr results in marked loss of inhibitory protency. The typical double amphiphilic structure of sequences containing a basic region separated from a region of mixed charges by an uncharged, hydrophobic region would appear to be important in the function of signals (Austen & Ridd, 1981). The tryptic fragments of the consensus sequence do not possess this double amphiphilic structure. The reduced inhibition of processing by these fragments may result from the hydrophobic nature of the fragment peptide containing residues 3–15, and it is noteworthy that Inouye *et al.* (1982), have recently shown that change of basic residues at the N-terminus of the signal of a bacterial membrane prelipoprotein slows, but does not prevent, assembly of the processed form of this protein into mem-
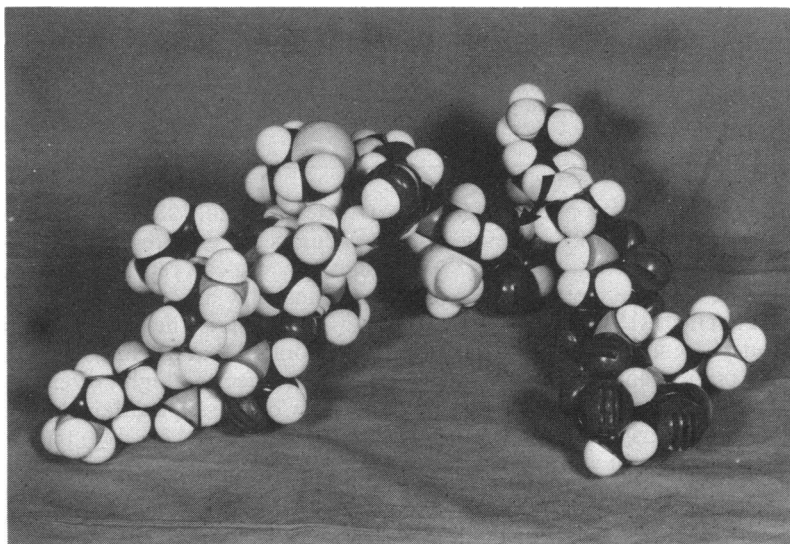

Fig. 6. *Molecular model of the consensus signal peptide*
The 19-residue peptide, built with CPK precision space-filling models, is shown. Residues 1–11 are α-helical, and a β-turn exists between residues 12 and 15. The hydrogen bond between the carbonyl oxygen of Cys to the amido nitrogen of Ala is marked with an arrow, holding the tetrapeptide sequence in a type 1 β-turn, which produces a reversal in the direction of the polypeptide chain.

branes. γ-Endorphin, which also possesses a double amphiphilic structure, but is different in that it contains two short stretches of hydrophobic uncharged residues separated by charged residues positioned in the centre of the peptide, is only weakly inhibitory. The lack of significant inhibition by human PTH-(28–48)-peptide shows that pronounced hydrophobicity in a relatively long uncharged sequence (11 residues) is insufficient in the absence of the typical charge distribution and potential for folding incorporated in the consensus sequence. Human PTH-(28–54)-peptide has previously been shown not to block processing of preprolactin (Majzoub *et al.*, 1980).

Predictions of conformation, and calculations of conformational energy, have shown that signal sequences are remarkably similar, despite differences in primary structure (Pincus & Klausner, 1982; Austen, 1979; Garnier *et al.*, 1980), and that their internal cores of uncharged hydrophobic residues are likely to adopt α-helical conformation, terminated by a β-turn. These empirical considerations have been supported by chiroptical measurements on synthetic peptides (Rosenblatt *et al.*, 1980), and it has been shown that an antibody directed against a particular signal peptide can recognize other signal peptides, possibly by acting as a conformational probe (Baty & Lazdunski, 1979). It is likely that, in the course of evolution, conformation of signals has been conserved rather than primary sequence. The consensus sequence has been designed to optimize the potential for folding into the common preferred conformation, shown in a space-filling model in Fig. 6. The calculated potential for α-helix and β-turn in the sequence (Chou & Fasman, 1978) is depicted in Fig. 1. The model represents the type of structure that is recognized by the cytoplasmic surface of endoplasmic reticulum in secretory cells. The nature of the components in these membranes that interact with signals has not been elucidated, but this soluble signal peptide should provide a useful probe for further studies.

References

Austen, B. M. (1979) *FEBS Lett.* **103**, 308–313

Austen, B. M. & Ridd, D. H. (1981) *Biochem. Soc. Symp.* **46**, 235–258

Austen, B. M. & Ridd, D. H. (1983) *Biochem. Soc. Trans.* **11**, 160–161

Bantle, J. A., Maxwell, I. H. & Hahn, W. E. (1976) *Anal. Biochem.* **72**, 413–427

Baty, D. & Lazdunski, C. (1979) *Eur. J. Biochem.* **102**, 503–507

Blobel, G. & Dobberstein, B. (1975) *J. Cell Biol.* **67**, 852–862

Blobel, G., Walter, P., Chang, C. N., Goldman, B. M., Erikson, A. H. & Lingappa, V. R. (1979) *Symp. Exp. Biol.* **33**, 9–36

Chan, S. J., Patzelt, C., Duguid, J. R., Quinn, P., Labreque, A., Boyes, B., Keim, P., Heinrikson, R. L. & Steiner, D. F. (1979) *Miami Winter Symp.* **16**, 361–378

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299

Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47**, 251–276

Devillers-Thiery, A., Kindt, T., Scheele, G. & Blobel, G. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5016–5020

Erickson, B. W. & Merrifield, R. B. (1976) *Proteins 3rd Ed.* **2**, 349–351

Garnier, J., Gaye, P., Mercier, J.-C. & Robson, B. (1980) *Biochimie* **62**, 231–239

Gisin, B. (1973) *Helv. Chim. Acta* **56**, 1476–1482

Inouye, S., Soberon, X., Franceschini, T., Nakamura, K., Itakura, K. & Inouye, M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3438–3441

Jackson, R. J. & Hunt, T. (1983) *Methods Enzymol.* **96**, 50–74

Kaiser, E., Colescott, R. L., Bossinger, C. D. & Cook, P. I. (1970) *Anal. Biochem.* **34**, 595–598

Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341

Majzoub, J. A., Rosenblatt, M., Fennick, B., Maunus, R., Kronenberg, H. M., Potts, J. T. & Habener, J. F. (1980) *J. Biol. Chem.* **255**, 11478–11483

Meyer, D. I., Krause, E. & Dobberstein, B. (1982) *Nature (London)* **297**, 647–650

Muller, M., Ibrahimi, I., Chang, C. N., Walter, P. & Blobel, G. (1982) *J. Biol. Chem.* **257**, 11860–11863

Palmiter, R. D., Gagnon, J. & Walsh, K. A. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3413–3417

Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256

Pincus, M. R. & Klausner, R. D. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3413–3417

Ridd, D. H., Hermon-Taylor, J. & Austen, B. M. (1982) *Biochem. Soc. Trans.* **10**, 46–47

Rosenblatt, M., Beudette, N. V. & Fasman, G. D. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3983–3987

Scheele, G., Jacoby, R. & Carne, T. (1980) *J. Cell Biol.* **87**, 611–628

Segrest, J. P. & Feldman, R. J. (1974) *J. Mol. Biol.* **87**, 835–838

Shields, D. & Blobel, G. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2059–2063

Szczesna, E. & Boime, I. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1179–1183

Walter, P. & Blobel, G. (1981) *J. Cell Biol.* **91**, 557–561